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TITLE: The Use of Venezuelan Equine Encephalitis Replicons Encoding the Her-2/neu Tumor Associated Antigen for the Prevention and Treatment of Breast Cancer

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Overview:

During the past year, we have been focusing on the objectives outlined for Specific Aim 1 in our approved statement of work, and have completed an initial experiment related to Specific Aim 2. Testing and packaging of VEE replicon constructs encoding Her-2/neu, IL-12 and IL-18 have been completed. Additionally, a replicon construct encoding murine granulocyte-macrophage colony stimulating factor (mGM-CSF), has been established and tested and a truncated Her-2/neu gene encoding the extracellular and transmembrane domain of the protein is currently being assessed. We are hopeful the reduced size of theHer-2/*neu* gene will lead to increased levels of RNA and protein expression.

The rat Her-2/neu gene encodes for an 185kDa transmembrane protein. In order that we may have protein to work with experimentally, we have cloned overlapping fragments of the gene into a bacterial expression plasmid. Currently, one protein generated from the bacterial expression system, encompassing parts of the extracellular and transmembrane domains, has been used to demonstrate $CD4^+$ T helper (Th) cell proliferation (Figure 1). For this experiment, mice were immunized intraperitoneally with 100μg of the protein fragment emulsified in incomplete Freund's adjuvant (IFA). Splenocytes were then recovered and assessed for T cell proliferation by ^{3}H-thymidine uptake. Both rat HER-2/*neu* and A2-K^b transgenic mice showed significant proliferative responses. A second fragment, further spanning the extracellular domain, is in the process of being tested.

We have begun to characterize the $CD8^+$ T cell response induced by replicons encoding Her-2/*neu* in both wild type FVB, and in FVB mice transgenic for the rat HER-2/*neu* gene. We have repeatedly generated significant CTL responses in wild type FVB mice, but CTL reactivity induced in Her-2/*neu* transgenic mice has to date been limited (Figure 2). The work of Jaffee et al. has demonstrated that Neu-specific T cell responses can be generated in Her-2/*neu* transgenic mice immunized with recombinant vaccinia virus expressing Neu.[1] Consequently, we are hopeful that the appropriate combination of replicons encoding Her-2/*neu* as well as key inflammatory cytokines will overcome this element of immune tolerance seen in the transgenic mice.

One experiment has been completed in which both wild type FVB and Her-2/*neu* transgenic mice were immunized with replicons encoding Her-2/*neu*, and subsequently challenged with the F-H2N1 tumor cell line (expressing Her-2/*neu*). Both immunized and unimmunized Her-2/neu transgenic mice exhibited significant tumor growth whereas both groups of wild type mice rejected the tumor. While there was no significant difference in tumor weight from immunized and unimmunized Her-2/*neu* transgenic mice, decreased vascularity was noted in tumors recovered from the VEE Her-2/*neu* immunized mice. While we have no evidence that this decreased vascularity may be attributable to the vaccination, we remain optimistic that the correct combination of antigen and cytokine encoding replicons will be effective in inhibiting tumor growth.

Many of the tools are now in place that will allow us to test various antigen and cytokine combinations for optimal $CD4^+$ Th and $CD8^+$ CTL reactivity. This will further allow us to establish an effective approach of immunotherapy targeting Her-2/*neu* for the treatment and prevention of breast adenocarcinoma in transgenic mice.

Key Accomplishments:

- Completed *in vitro* testing and packaging of the following VEE replicons:

  Full length Her-2/neu
  IL-12
  IL-18
  mGM-CSF
  IL-2

- Currently testing truncated Her-2/*neu* replicon encoding the extracellular and transmembrane domains.

- Cloned fragments of the Her-2/*neu* gene into a bacterial expression system and generated recombinant protein.

- Demonstrated $CD4^+$ Th cell reactivity to one Her-2/*neu* protein fragment, and are in the process of testing a second fragment.

- Repeatedly generated $CD8^+$ CTL reactivity in wild type FVB mice.

- Completed one tumor challenge experiment in VEE Her-2/*neu* immunized mice.

Reportable Outcomes:

- None.

Figures and Tables:

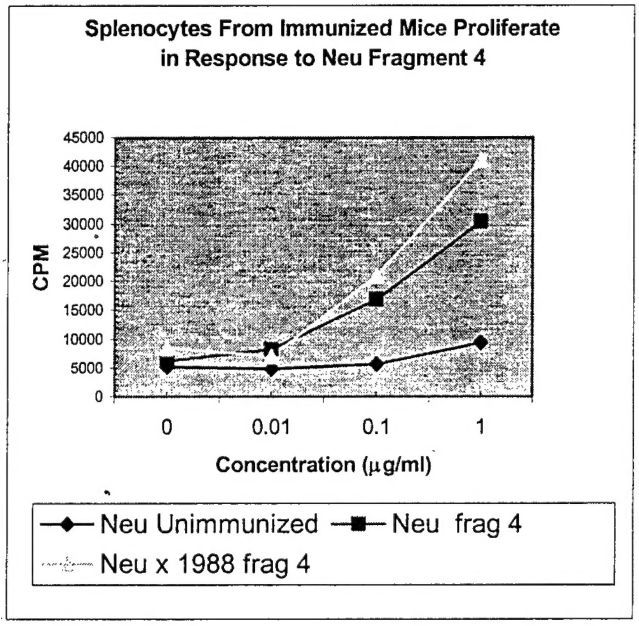

Figure 1. Proliferation in response to Her-2/*neu* fragment 4. Fragment 4 encompasses nucleotides 1856 – 2585 spanning part of the extracellular domain, the entire transmembrane domain and a small section of the intracellular domain. Her-2/neu transgenic and A2-K^b transgenic mice were twice immunized intraperitoneally with 100 µg fragment 4 emulsified in IFA. Splenocytes were harvested and incubated with the indicated concentration of recombinant protein and the levels of ^{3}H-thymidine incorporation were measured. Data represents groups of three mice.

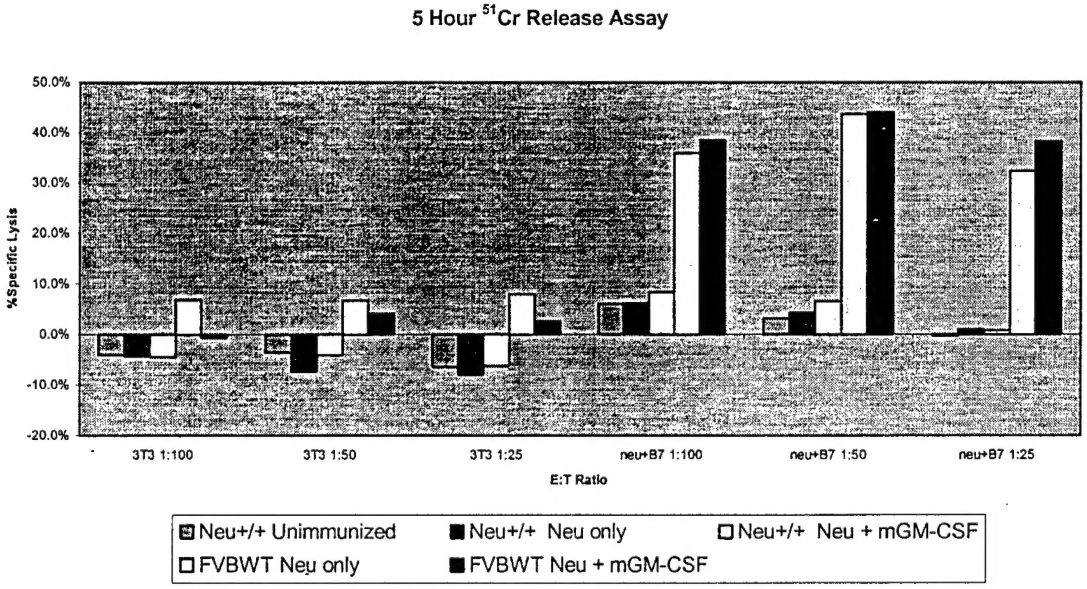

Figure 2. Standard 5 hr chromium release assay for FVB and FVB/$Neu^{+/+}$ mice immunized with VEE replicons encoding Her-2/*neu* ($3.0x10^5$ IU) and replicons encoding mGM-CSF ($3.0x10^4$ IU) . Mice were immunized in the footpad 3X at 7 day intervals and sacrificed 3 – 5 days following the final injection. Spleen and popliteal lymph node cell suspensions were stimulated *in vitro* with psorelen inactivated NIH-3T3 cells ($H\text{-}2^q$) permanently transfected with Her-2/*neu* and B7-1 encoding constructs. Stimulated lymphocytes were incubated with ^{51}Cr labeled targets in the indicated ratios for 5 hours. 3T3 = non-transfected NIH-3T3 cells. Neu+B7 = NIH-3T3 cells transfected with Her-2/*neu* and B7.1. Data represents groups of 2 mice.

Summary:

Much of the work described for Specific Aim 1 has now been completed. We have finished the *in vitro* testing and packaging of the replicon constructs described, and have begun work on additional constructs. Progress has been made characterizing the $CD8^+$ T cell response in both wild type and Her-2/*neu* transgenic mice with generation of consistent CTL activity in the wild type mice. Additionally, we have cloned overlapping fragments of the Her-2/*neu* gene into a bacterial expression system, and demonstrated a proliferative response to one of the recombinant proteins. These fragments will be used to evaluate $CD4^+$ T helper responses in immunized mice. We believe an immunotherapeutic protocol consisting of the appropriate combination and antigen and cytokine encoding replicons will be useful for overcoming the tolerance associated with the Her-2/neu transgenic mice.

In our initial tumor challenge experiment with replicons encoding Her-2/*neu* alone, we failed to mediate rejection of transferred Neu expressing tumors in Her-2/*neu* transgenic mice. Again, we feel it will be a matter of determining the appropriate cytokine encoding constructs for use in our protocol.

References:

Reilly, R. Todd, Morris B.C. Gottlieb, Anne M. Ercolini, Jean-Pascal H. Machiels, Colin E. Kane, Francesca I. Okoye, William J. Muller, Katherine H. Dixon, and Elizabeth M. Jaffee. (2000). Her-2/*neu* Is a Tumor Rejection Target in Tolerized HER-2/*neu* Transgenic Mice. Cancer Research 60, 3569-3576